A non-native invasive grass increases soil carbon flux in a Hawaiian tropical dry forest

CREIGHTON M. LITTON*, DARREN R. SANDQUIST* and SUSAN CORDELL†

*Department of Biological Science, California State University Fullerton, 800 N State College Blvd, Fullerton, CA 92834, USA,
†USDA Forest Service, Institute of Pacific Islands Forestry, 60 Nowelo St, Hilo, HI 96720, USA

Abstract

Non-native plants are invading terrestrial ecosystems across the globe, yet little is known about how invasions impact carbon (C) cycling or how these impacts will be influenced by climate change. We quantified the effect of a non-native C$_4$ grass invasion on soil C pools and fluxes in a Hawaiian tropical dry forest over 2 years in which annual precipitation was average (Year 1) and ~60% higher than average (Year 2). Work was conducted in a series of forested plots where the grass understory was completely removed (removal plots) or left intact (grass plots) for 3 years before experiment initiation. We hypothesized that grass invasion would: (i) not change total soil C pools, (ii) increase the flux of C into and out of soils, and (iii) increase the sensitivity of soil C flux to variability in precipitation. In grass plots, grasses accounted for 25–34% of litter layer C and ~70% of fine root C. However, no differences were observed between treatments in the size of any soil C pools. Moreover, grass-derived C constituted a negligible fraction of the large mineral soil C pool (<3%) despite being present in the system for ≥50 years. Tree litterfall was ~45% lower in grass plots, but grass-derived litterfall more than compensated for this reduction in both years. Annual cumulative soil-surface CO$_2$ efflux ($R_{\text{soil}}$) was ~40% higher in grass plots in both years, and increased in both treatments by ~36% in the wetter Year 2. Despite minimal grass-derived mineral soil C, >75% of $R_{\text{soil}}$ in grass plots was of C$_4$ (i.e. grass) origin. These results demonstrate that grass invasion in forest ecosystems can increase the flux of C into and out of soils without changing total C pools, at least over the short term and as long as the native tree canopy remains intact, and that invasion-mediated changes in belowground C cycling are sensitive to precipitation.

Keywords: belowground carbon cycling, Hawaii, invasive species, litterfall, litter decomposition, Pennisetum setaceum ('fountain grass'), soil carbon pools and fluxes, soil-surface CO$_2$ efflux ('soil respiration')

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Introduction

Non-native plants are invading nearly all terrestrial ecosystems and are among the most pervasive elements of global change biology (Vitousek et al., 1997; Mack et al., 2000; Didham et al., 2005). Previous work has documented that invasions can have substantial impacts on both the structure and function of native ecosystems (see Ehrenfeld, 2003). Most work to date has concentrated on nutrient dynamics, but plant invasions also have the potential to alter the terrestrial carbon (C) cycle at local to regional scales (Litton et al., 2006). However, few studies have examined how invasive species impact C transfers across the soil–plant–atmosphere interface. Moreover, interactions between invasive species and climate have yet to be explored within the context of C cycling.

Understanding the impacts of plant invasions on belowground C cycling is critical for predicting how invasive species will affect the exchange of C between the atmosphere and terrestrial ecosystems. Soil-surface CO$_2$ efflux ($R_{\text{soil}}$; 'soil respiration') is the largest flux of
The addition of a new species via invasion has the potential to fundamentally alter ecosystem processes. Changes in species abundance and diversity can strongly influence ecosystem function because individual species differ in the rate at which resources are acquired, the efficiency with which those resources are utilized to produce biomass, and interactions with the physical environment and other organisms (Chapin et al., 1997). Non-native invasive species most likely to alter ecosystem processes are those that: (i) differ from natives in resource acquisition and utilization, (ii) alter trophic structures, or (iii) influence disturbance regimes (Vitousek, 1990). The success of invasive plants has been at least partially linked to all three of these mechanisms (Mack et al., 2000; Daehler, 2003; Callaway & Ridenour, 2004).

Island ecosystems are particularly susceptible to plant invasions (Gimeno et al., 2006). In Hawaii, it is estimated that <10% of the original tropical dry forest remains (Bruegmann, 1996), primarily as a result of the introduction of invasive species and changes in disturbance regimes (Hughes et al., 1991; D’Antonio & Vitousek, 1992). Native grasses were presumably a minor component of this system, but today existing fragments of dry forest on the Island of Hawaii are often heavily invaded by the non-native C4 perennial bunchgrass Pennisetum setaceum (Forssk.) Chiov. (*fountain grass*). Previous work in these forest fragments has documented that grass invasion into otherwise intact native dry forest increases understory biomass ~7× but has no impact on total aboveground C pools because of the large amount of woody biomass in trees (Litton et al., 2006). However, there is often poor correlation between biomass and C flux in forests (Litton et al., 2007), and information on both C pools and fluxes is needed to develop a comprehensive understanding of how invasive species impact C cycling.

Here, we use *P. setaceum* invasion into Hawaiian tropical dry forest to address how a non-native invasive plant impacts belowground C cycling by examining soil C pools and fluxes in the presence and absence of grass invasion. Specifically, our objectives were to compare plots invaded by *P. setaceum* and plots where the grass had been removed for 3 years before experiment initiation to quantify: (i) C pools in the litter layer, roots, microbial biomass and mineral soil, (ii) C fluxes in *R*soil, aboveground litterfall and litter decomposition, (iii) the relationship between *R*soil and leaf area index (LAI), and (iv) the C3 vs. C4 contributions to soil C pools and fluxes. In addition, our measurements span a 2-year time period in which annual rainfall differed by ~60%, allowing us to explore how variation in precipitation interacts with plant invasion to impact belowground C cycling. We hypothesized that: (H1) grass invasion would result in minimal changes in the size of soil C pools (Ehrenfeld, 2003), as loss of forest C3-C would be replaced by new grass C4-C (Bashkin & Binkley, 1998; Binkley & Resh, 1999; Osher et al., 2003); (H2) the flux of C into and out of soils would increase with invasion, and the grass contribution to these fluxes would be proportional to its contribution to total ecosystem C pools; and (H3) increased precipitation would increase all components of soil C flux, both with and without grass, but the relative increase in flux would be higher in grass-invaded forests (i.e. increased sensitivity to changes in precipitation with grass invasion) because grass production would be more limited by near-surface soil water availability.

Materials and methods

Study area

The study was carried out in the Kaupulehu Dry Forest Preserve (608 m elevation, 19°46′05″N, 155°56′19″W), located on the northwest slope of Hualalai Volcano in the district of North Kona, Island of Hawaii (see Litton et al., 2006). Based on aerial photography, *P. setaceum* has been a dominant component of this landscape since at least the early 1960s, and likely decades earlier. Experimental plots were located in a 7–8 m tall mature forest that is dominated by two native evergreen trees, Diospyros sandwicensis (A. DC) Fosb. and Psydrax odorata (Forst. F.) A. C. Sm. and S. Darwin, which together account for >91% of the total basal area and stem density (Litton et al., 2006).

Parent material of the study site consists of tholeiitic basalt laid down primarily as ‘a’a lava flows (rough and blocky) ~1500–3000 years ago (Moore et al., 1987). Soils are shallow, highly organic (fine fraction of mineral soil >30% C), >50% coarse rocks (>5 mm) by volume, with an average pH of 7.6 (± 0.1), and are classified as euic, isothermic, shallow, Lithic Ustisol
(USDA-NRCS classification). The high soil C content and vegetation characteristics suggest that our study area occurs on the upper end of the estimated substrate age range (see Stemmermann & Ihsle, 1993), and may well be older.

Long-term mean annual rainfall for the study area is ~700–750 mm, with great year-to-year variability (Giambelluca et al., 1986; Litton et al., 2006). Mean annual rainfall from 1999 to 2005 was 514 mm, with a low of 225 mm in 1999 and a high of 1249 mm in 2004. Large rainfall events can occur during any month, but summers tend to be drier than winters. Total annual precipitation was 732 mm during Year 1 of this study (May 1, 2003 to April 30, 2004) and 1189 mm during Year 2 (May 1, 2004 to April 30, 2005). At a nearby weather station (~10 km; 709 m elevation; 19°47′42″N, 155°50′43″W), mean daily temperature for 2004–2005 was 20.0 °C, with a mean daily maximum of 25.6 °C and a mean daily minimum of 15.1 °C.

Experimental design

Work was conducted in a series of eight forested plots (12 m × 12 m) that were randomly established in July 2000 – the grass understory was removed from half of the plots (removal plots) and left intact in the remaining four plots (grass plots). Tree basal area and stem density did not differ between treatments (P > 0.78; Table 1). In June 2003, an additional 4 m buffer was established around plots to minimize edge effects (20 m × 20 m plots with all measurements taken in the central 12 m × 12 m). Grass was removed from removal plots and buffer areas with a general herbicide and manual extraction of aboveground biomass (Cordell et al., 2002). Grass seedlings were pulled from removal plots on a regular basis. At the start of the study, 3 years after plot establishment, the understory in grass plots was a monospecific cover of *P. setaceum*, while the understory in removal plots consisted of scattered native shrub, vine and graminoid species which established following grass removal [*Lipochaeta subcordata* A. Gray; *Cocculelus trilobus* (Thunb.) DC; *Sicyos lasiocephalus* Skotts.; and *Mariscus hillebrandii* (Boeck.) T. Koyama].

**Table 1** Stand characteristics of the tropical dry forest study site on the Island of Hawaii with (grass) and without (removal) *Pennisetum setaceum*, a non-native invasive grass

<table>
<thead>
<tr>
<th></th>
<th>Removal</th>
<th>Grass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal area (cm² m⁻²)</td>
<td>20.8 (1.4)⁵</td>
<td>22.0 (3.5)⁵</td>
</tr>
<tr>
<td>Stem density (trees ha⁻¹)</td>
<td>3420 (915)⁴</td>
<td>3368 (569)⁴</td>
</tr>
<tr>
<td>LAI (m² m⁻²) – Year 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overstory (&gt;1.33 m)</td>
<td>1.89 (0.16)⁴</td>
<td>1.37 (0.12)⁴</td>
</tr>
<tr>
<td>Understory (&lt;1.33 m)</td>
<td>0.21 (0.10)³</td>
<td>1.67 (0.18)³</td>
</tr>
<tr>
<td>LAI (m² m⁻²) – Year 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overstory (&gt;1.33 m)</td>
<td>1.86 (0.17)⁴</td>
<td>1.30 (0.10)⁴</td>
</tr>
<tr>
<td>Understory (&lt;1.33 m)</td>
<td>0.21 (0.10)³</td>
<td>2.38 (0.35)³</td>
</tr>
</tbody>
</table>

All values are treatment means (±SE; n = 4); means with the same letter within a row do not differ at z = 0.05. LAI, leaf area index.
preparation of soil-surface CO2 efflux

Soil-surface CO2 efflux

Soil-surface CO2 efflux ($R_{soil}$) was measured monthly at eight points in each plot with a LI-COR 6400 portable photosynthesis system and LI-COR 6400-09 soil respiration chamber (LI-COR, Lincoln, NE, USA). We used permanently placed 10 cm diameter polyvinyl chloride (PVC) collars inserted 4 cm into the litter and mineral soil. Two measurements were made at each point by drawing down chamber CO2 concentration below ambient concentrations at the soil surface, and measuring chamber CO2 concentration from 10 ppm below ambient to 10 ppm above ambient.

We quantified the temperature sensitivity and diel course of $R_{soil}$ by measuring efflux on all eight collars in one plot of each treatment every 3 h for 24 h in July 2004. In addition, we quantified the effects of discrete pulse precipitation events on $R_{soil}$ by measuring efflux 2 days before a large pulse event (95 mm), 1 day after the pulse event, and every 2–3 days thereafter for a month on all eight collars in one plot of each treatment.

To explore the relationship between $R_{soil}$ and environmental variables, we quantified soil water potential ($\Psi_{soil}$) and soil temperature throughout the experiment in three plots of each treatment. $\Psi_{soil}$ at 10 cm was measured weekly at four locations per plot using individually calibrated in situ thermocouple psychrometers (PST-55) and a microvolt meter (HR-33 T) operating in dew point depression mode (Wescor, Logan, UT, USA). Continuous, hourly measurements of soil temperature at 10 cm were made on three locations per plot with high-resolution temperature sensors (TMC6-HD; Onset Computer Corporation, Bourne, MA, USA).

Annual cumulative $R_{soil}$ (g C m$^{-2}$ yr$^{-1}$) was estimated using two approaches that rely on linear interpolation to fill in missing daily values. In the ‘monthly interpolation’ approach we used linear interpolation between monthly $R_{soil}$ measurements, while in the ‘weekly interpolation’ approach we supplemented monthly $R_{soil}$ measurements with weekly $R_{soil}$ estimates based on the relationship between $\Psi_{soil}$ and $R_{soil}$ (see ‘Results’). $R_{soil}$ exhibited no temperature or diel response (see ‘Results’), so measured instantaneous rates ($\mu$mol CO2 m$^{-2}$ s$^{-1}$) were scaled to daily C flux rates (g C m$^{-2}$ day$^{-1}$) with no temperature or diel correction.

Aboveground litterfall and litter decomposition

Tree aboveground litterfall was collected monthly in all plots from eight 0.1764 m$^2$ traps. Litter from each trap was dried at 70 °C to a constant mass in a forced air oven and weighed. The C content of litterfall was analyzed as above on a subset of composite samples, and the mean value (43.7%) was used to quantify aboveground litterfall C flux. For grass and all other understory vegetation, annual litterfall was assumed equivalent to annual aboveground net primary production, which was estimated by summing positive increments in live and dead standing biomass (trough-peak analysis) in grass plots (grasses), and with clip plots during peak standing biomass in removal plots (all other understory vegetation) (Scurlock et al., 2002). Grass biomass was 44.5% C.

Litter decomposition of D. sandwicensis and P. setaceum foliage was assessed in situ for 10 months with 100 cm$^2$ litterbags made of 2 mm fiberglass mesh. Recently senesced foliage was collected in all plots, air dried and composited by species and treatment. A well-mixed subsample of each species and treatment combination was dried to a constant mass in a forced air oven to determine percent moisture for correcting initial litter mass. Approximately 1.5 g of intact, air-dried litter was weighed and placed in each litterbag, and four litterbags of each species and treatment combination were placed in plots in March, 2004 (D. sandwicensis in removal and grass plots, and P. setaceum in grass plots). Initial litter chemistry (% C, N, lignin, cellulose and hemicellulose) was determined on a well-ground subsample of each litter type and treatment combination using sequential C fraction analysis (Ryan et al., 1990). Litterbags were collected after 10 months, soil was carefully removed from each sample, samples were dried to a constant mass, weighed, and species replicates within a given plot were composited, ground in a Wiley mill, and analyzed for percent ash in a muffle furnace and CHN content on a Costech ECS 4010 Elemental Combustion Analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA).

$C_4$ contribution to soil C pools and fluxes

The grass contribution ($C_4$ grass) to soil C pools in the research area ($C_3$ forest) was assessed using a standard two-member isotope mixing model of the form

$$f_{\lambda} = \frac{\delta_M - \delta_B}{\delta_{\lambda} - \delta_B},$$

where $f_{\lambda}$ is the fraction of carbon in the source (litter) that is $C_4$. 

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where $\delta_M$, $\delta_A$, and $\delta_B$ are the mean $\delta^{13}C$ isotopic signatures of the mixture (M) and sources A and B, respectively, and $f_A$ is the proportion of A in M (Phillips & Gregg, 2001). The model was run separately for the litter layer, roots and mineral soil fine fraction. End members (A and B) for each mixing model came from measured $\delta^{13}C$ values of: (i) litter layer C – P. setaceum and tree litter; (ii) root C – P. setaceum and tree roots; and (iii) mineral soil C – P. setaceum roots and preinvasion soil organic matter (no C$_4$ contribution) calculated as the $\delta^{13}C$ signature of tree foliage enriched by 2%o. The 2%o enrichment assumption for mineral soil end member designation is based on: (i) a nearby (<5 km), uninvaded dry forest with similar vegetation (see Litton et al., 2006), where the isotopic signature of bulk mineral soil is 2.04%o enriched compared with in situ tree foliage (C. M. Litton, unpublished data); and (ii) the commonly observed phenomenon in forest ecosystems that mineral soil is enriched 1–3%o compared with foliage (Ehleringer et al., 2000).

We also quantified the grass (C$_4$) contribution to $R_{soil}$ using a Keeling plot approach (Pataki et al., 2003) on two locations in one plot of each treatment in July of 2004. A custom soil chamber (0.02 m$^3$) was placed over the soil surface and sealed with an O-ring made from nylon hose filled with fine sand. Soil chamber CO$_2$ concentrations were determined by circulating air through the chamber with a LI-COR 6400 portable photosynthesis system. Small samples of soil chamber air were extracted periodically via an inline septum using a 10 mL syringe over 5–10 min as chamber CO$_2$ concentration increased from ambient to ~550 ppm. Samples were stored in 10 mL Exetainer vials (Labco, UK) that had been flushed and filled with He. All samples were analyzed for $\delta^{13}C$ within 60 h of field sampling on a Finnigan MAT Delta-S isotope ratio mass spectrometer with precon at the Stable Isotope Ratio Facility for Environmental Research (University of Utah). The $y$-intercept of the resulting least squares linear regression between $\delta^{13}C$ (y-axis) and the inverse of CO$_2$ concentration (1/[CO$_2$]; x-axis), the Keeling plot (Keeling, 1958), predicts the $\delta^{13}C$ value of CO$_2$ entering the chamber from the soil. A two-member mixing model was used to determine the C$_4$ and C$_3$ contributions to $R_{soil}$ (Phillips & Gregg, 2001). End members in the mixing model were measured $\delta^{13}C$ values: grass roots for the C$_4$ component and grass-free forest mineral soil and tree roots for the C$_3$ component. For the latter end member, we assumed that 50% of $R_{soil}$ was autotrophic (root, root exudates, mycorrhizae) and 50% heterotrophic (mineral soil) origin (see Bond-Lamberty et al., 2004), such that the C$_3$ end member was calculated as the average of root and mineral soil $\delta^{13}C$.

Contribution to aboveground litterfall by trees (C$_3$) and grasses (C$_4$) was quantified by simply dividing litterfall flux of grasses or trees by total aboveground litterfall (tree + grass).

**Leaf area index**

LAI (m$^2$ m$^{-2}$) was estimated in August of both years for the overstory and understory between 4:00 and 6:00 hours on days with widespread cloud cover. Understory LAI was estimated with a pair of LAI-2000 plant canopy analyzers (Li-Cor Inc., Lincoln, NE, USA), where diffuse solar radiation was measured below and above the canopy simultaneously. Below canopy measurements were taken directly above the understory vegetation at 1.33 m on eight points per plot on a systematic grid. For understory LAI, we used the LAI-2000 operating in 1 unit mode, by taking measurements at the soil surface and at 1.33 m. We corrected understory LAI estimates to include only live foliage (LAI multiplied by %biomass in live tissues) by harvesting standing grass biomass from fifteen 0.25 m$^2$ plots and quantifying the live:dead biomass ratio, with the assumption that live and dead foliage had similar mass per area, as well as reflective and transmittance properties. Field data were analyzed using fV2000 software masking the outermost ring (Li-Cor Inc.). No corrections were made to account for woody biomass in the canopy and, therefore, our overstory LAI values refer to plant area, and likely overestimate leaf area (Kalacska et al., 2005).

**Statistical analysis**

The sample unit for this study was the plot and all data presented are mean plot values ($n = 3$ or $n = 4$ depending on the measurement). All statistical analyses were performed in SPSS 10.0 for WINDOWS (SPSS Inc., Chicago, IL, USA) at an $\alpha = 0.05$. All data were tested for assumptions of normality and equality of variance. Tests for differences among treatments in stand structural characteristics, soil C pools, and the C$_4$ contribution to soil C pools and fluxes were performed using one-way analysis of variance (ANOVA) – except for the C$_4$ contribution to $R_{soil}$, where treatment differences were analyzed with an independent sample $t$-test. Treatment and time effects for $R_{soil}$ (instantaneous, daily and yearly), $F_A$ (monthly and yearly), soil temperature, and $\Psi_{soil}$ were analyzed with a repeated measures general linear model. Linear and nonlinear regression techniques were used to explore the relationships between $R_{soil}$ and soil temperature, soil water potential, and LAI.
Table 2  Soil carbon pools (g C m⁻² to 30 cm depth) in grass and grass removal plots

<table>
<thead>
<tr>
<th>Component</th>
<th>Removal (g C m⁻²)</th>
<th>Grass (g C m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litter layer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year 1</td>
<td>548 (75)</td>
<td>592 (77)</td>
</tr>
<tr>
<td>Year 2</td>
<td>291 (12)</td>
<td>327 (60)</td>
</tr>
<tr>
<td>Roots</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fine (&lt;2 mm)</td>
<td>209 (36)</td>
<td>191 (49)</td>
</tr>
<tr>
<td>Coarse (≥ 2 mm)</td>
<td>144 (50)</td>
<td>145 (47)</td>
</tr>
<tr>
<td>Total root</td>
<td>353 (85)</td>
<td>336 (93)</td>
</tr>
<tr>
<td>Mineral soil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fine (&lt;2 mm)</td>
<td>20695 (3114)</td>
<td>20281 (1897)</td>
</tr>
<tr>
<td>Coarse (≥ 2 mm, &lt;5 mm)</td>
<td>610 (271)</td>
<td>524 (67)</td>
</tr>
<tr>
<td>Rock*</td>
<td>467 (98)</td>
<td>624 (135)</td>
</tr>
<tr>
<td>Cmic*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year 1</td>
<td>1.9 (0.3)</td>
<td>1.9 (0.1)</td>
</tr>
<tr>
<td>Year 2</td>
<td>1.9 (0.1)</td>
<td>1.9 (0.5)</td>
</tr>
<tr>
<td>Total mineral soil</td>
<td>21771 (2948)</td>
<td>21428 (1936)</td>
</tr>
<tr>
<td>Total soil carbon</td>
<td>22627 (2855)</td>
<td>22356 (1772)</td>
</tr>
</tbody>
</table>

Values are treatment means (±1SE; n = 3); means with the same letter within a row do not differ at z = 0.05.
*Organic matter (roots and mineral soil C) contained within porous lava rocks.
†Microbial biomass C (mg C g dry soil⁻¹).

Results

Soil C pools

No differences were observed in any soil C pools between removal and grass plots 3–5 years after grass removal (P > 0.39; Table 2). Total soil C was ~22 500 g C m⁻² to 30 cm, and was dominated by the mineral soil fine fraction which accounted for 91% of total soil C in both treatments despite only accounting for 9% of soil mass and ~35% of soil volume. Of the remaining C, 5% was in rocks and the mineral soil coarse fraction, while litter and fine roots accounted for an additional 1–2% each. For pools measured in both years, litter C was ~45% smaller in the wetter Year 2 (P < 0.03) and Cmic did not differ between years (P = 0.99).

Soil-surface CO₂ efflux

Instantaneous measurements of Rsoil were higher in grass plots (P < 0.01; Fig. 1a). Across both treatments Rsoil ranged from 0.81 to 7.12 µmol CO₂ m⁻² s⁻¹, with overall means of 3.09 and 4.39 in removal and grass plots, respectively. Rsoil varied with time (P < 0.01) and displayed a significant time-by-treatment interaction (P < 0.01). Soil temperature was higher in removal plots (P = 0.01; Fig. 1b), and there was a distinct temporal pattern to temperature (P < 0.01) that differed by treatment (P = 0.02). Soil temperature peaked during the summer but varied by only ~5 °C throughout the year (Fig. 1b). Soil water potential also varied with time (P < 0.01), with driest conditions in the summer, but neither treatment nor time-by-treatment interactions were significant (P = 0.93 and 0.69, respectively; Fig. 1c).

For both grass and removal plots, there was no relationship between Rsoil and soil temperature at 10 cm (R² = 0.04; P > 0.10), and the slope of the relationship was negative for both treatments. There was also no significant diel fluctuation in Rsoil for either treatment (P > 0.50). For the 24 h measurement period, soil temperature varied by only 2 °C and Rsoil by <10% between maximum and minimum measured values.

In contrast, Rsoil closely followed seasonal patterns of Ψsoil (Fig. 1), although the strong seasonality of both...
was diminished in the wetter Year 2. There was a significant correlation between $R_{\text{soil}}$ and $C_{\text{soil}}$ for both removal and grass plots (Fig. 2). The relationship was best explained by an exponential model, which accounted for 59–66% of the variance in the data. Model fit was best at $C_{\text{soil}}/C_0^{0.5}$ MPa, as flux rates varied more when soils were at or near saturation. Because flux rates increased with grass invasion, the slope of the relationship between $R_{\text{soil}}$ and $C_{\text{soil}}$ was higher in grass plots.

$R_{\text{soil}}$ responded rapidly to a large precipitation pulse (95 mm), increasing by >75% within 24 h in both treatments (Fig. 3), despite the fact that soils were near saturation (−0.35 MPa) before the pulse event. Cumulative $R_{\text{soil}}$ based on linear interpolation between three monthly measurements over a 58-day period (Fig. 3; solid line) resulted in flux estimates that were 18–22% lower than that from linear interpolation between flux measured every 2–3 days (Fig. 3; dashed line). Cumulative flux from intensive measurements differed by 1–6% when linear interpolation of monthly efflux measurements was supplemented with efflux predictions based on weekly soil water potential measurements (weekly; dotted line).

Despite the large effect of pulse events on cumulative flux over short temporal scales, there was less difference on an annual scale between cumulative flux calculated with interpolation between monthly measurements and that calculated by supplementing monthly measurements with weekly estimates of $R_{\text{soil}}$ (0.5–7%; Table 3). Herein, we use annual cumulative $R_{\text{soil}}$ calculated from linear interpolation between monthly measurements supplemented with weekly estimates based on the flux vs. $C_{\text{soil}}$ relationship.

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**Fig. 2** Soil-surface CO$_2$ efflux ($R_{\text{soil}}$) was strongly related to soil water potential ($\Psi_{\text{soil}}$) ($P<0.01$) in both (a) removal plots and (b) grass plots over a wide range of $\Psi_{\text{soil}}$ observed during 2 years of measurements.

**Fig. 3** Daily soil-surface CO$_2$ efflux ($R_{\text{soil}}$; g C m$^{-2}$ day$^{-1}$) increased by >75% in both (a) removal plots and (b) grass plots within 3 days of (c) a large precipitation event. Linear interpolation between monthly efflux measurements (monthly; solid line) underestimated cumulative flux over this 58-day period by 18–22% compared with interpolation between measurements taken every 2–3 days (intensive; dashed line). Cumulative flux from intensive measurements differed by 1–6% when linear interpolation of monthly efflux measurements was supplemented with efflux predictions based on weekly soil water potential measurements (weekly; dotted line).
Annual cumulative $R_{soil}$ was 38–40% higher in grass plots over the course of the study ($P < 0.01$; Table 3), ranging from a low of 1024 g C m$^{-2}$ yr$^{-1}$ in removal plots in Year 1 to a high of 1943 g C m$^{-2}$ yr$^{-1}$ in grass plots in Year 2. $R_{soil}$ increased by $\sim 36\%$ in both treatments in the wetter Year 2 ($P < 0.01$). Annual $R_{soil}$ was positively and linearly correlated with total LAI (understory + overstory) across all plots and both measurement years (Fig. 4).

### Table 3 Cumulative annual flux of C from soils ($R_{soil};$ g C m$^{-2}$ yr$^{-1}$) in grass and grass removal plots

<table>
<thead>
<tr>
<th>Soil-surface CO$_2$ efflux (g C m$^{-2}$ yr$^{-1}$)</th>
<th>Removal</th>
<th>Grass</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monthly interpolation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year 1</td>
<td>1039 (59)$^a$</td>
<td>1318 (73)$^b$</td>
</tr>
<tr>
<td>Year 2</td>
<td>1322 (83)$^a$</td>
<td>1957 (118)$^b$</td>
</tr>
<tr>
<td><strong>Weekly interpolation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year 1</td>
<td>1024 (32)$^a$</td>
<td>1417 (24)$^b$</td>
</tr>
<tr>
<td>Year 2</td>
<td>1384 (24)$^a$</td>
<td>1943 (49)$^b$</td>
</tr>
</tbody>
</table>

Estimates are based on either linear interpolation between monthly efflux measurements (monthly), or linear interpolation between monthly efflux measurements supplemented with weekly efflux predicted from soil water potential measurements (weekly). Values are treatment means ($\pm$ 1 SE; $n = 4$); means with the same letter within a row do not differ at $\alpha = 0.05$.

### Aboveground litterfall and litter decomposition

Monthly aboveground tree litterfall was higher in removal plots ($P < 0.01$; Fig. 5a) and displayed distinct seasonality ($P < 0.01$) that differed by treatment ($P = 0.04$). Tree litterfall was lowest during the dry summer (Fig. 5a) and, overall, followed seasonal patterns of $R_{soil}$ (Fig. 1a). Annual aboveground tree litterfall was $\sim 2 \times$ higher in removal plots ($P < 0.01$; Fig. 5b), did not differ between years ($P = 0.40$), and exhibited no time-by-treatment interaction ($P = 0.70$). Understory litterfall was 16–44 times higher in grass plots ($P < 0.01$; Fig. 5b) and increased by 170% in Year 2 ($P < 0.01$). High understory litterfall in grass plots more than compensated for reduced tree litterfall in both years such that total aboveground litterfall was higher in grass plots ($P < 0.01$; Fig. 5b), with significant time ($P < 0.01$) and time-by-treatment interactions ($P < 0.01$).
Foliage litter decomposition of *D. sandwicensis*, the dominant native tree, was more rapid in grass plots than removal plots ($P < 0.02$), with 46% and 57% of the original mass remaining after 10 months, respectively (Fig. 6). Treatment differences existed in both microenvironment and initial litter chemistry. In grass plots, litter layer moisture (quantified gravimetrically in March 2005) was higher and % lignin was lower ($P < 0.01$) than in removal plots. In addition, leaf mass per area (LMA) and foliar C : N were higher, and tree leaf N concentration lower in grass plots ($P < 0.03$). Grass foliage in grass plots decomposed faster than tree foliage in both grass and removal plots ($P < 0.02$), with only 37% mass remaining after 10 months (Fig. 6).

**C$_4$ contribution to soil C pools and fluxes**

Grass contributed substantially to soil C pools for only the litter layer (25–34%) and fine roots (72%) in grass plots (Fig. 7). In removal plots, residual grass material contributed 11% to the fine root C pool (live + dead roots) and ~2% to litter layer C 4 years after removal. There was a negligible contribution of C$_4$ to the large mineral soil C pool in both treatments (~3%; Fig. 7). In contrast, the C$_4$-C contribution to flux in grass plots was 77% for $R_{soil}$ and 65% and 85% for aboveground litterfall in Years 1 and 2, respectively. In removal plots, there was no C$_4$ contribution to aboveground litterfall and a small contribution to $R_{soil}$ (10%; Fig. 7).

**LAI**

Overstory LAI was ~40% higher in removal plots than in grass plots ($P = 0.04$; Table 1) and did not vary across years ($P = 0.45$). Both understory and total (understory + overstory) LAI were higher in grass plots ($P < 0.01$) and increased in the wetter Year 2 of the study ($P = 0.01$), due exclusively to changes in understory LAI.

**Discussion**

Previous studies have shown that invasive grasses can impact soil nutrient (Mack, 2003; Mack & D’Antonio, 2003) and plant water dynamics (Ludwig *et al*., 2004). However, studies of invasive species’ impacts on belowground C cycling have been few, have primarily dealt with C pools, and have varied widely in the magnitude and direction of inferred impacts (Scott *et al*., 2001; Ehrenfeld, 2003; Hook *et al*., 2004). Grass invasion into this forest greatly increased the flux of C into and out of soils, as well as the composition of rapidly cycling C pools. However, minimal grass-derived C has been stabilized in soil organic matter, and after ~50 years in the system grass invasion has not altered the size or isotopic signature of the mineral soil C pool.

**Soil C pools and C$_4$ contribution**

Our estimate of total soil C in this system is ~4 x larger than C in aboveground live biomass (~55 Mg C ha$^{-1}$; Litton *et al*., 2006) and, thus, represents a very large and
important pool of ecosystem C. In agreement with our first hypothesis (H1), invasion by a $C_4$ perennial bunchgrass has not led to changes in the overall size of soil C pools even though non-native grass has been a dominant component of the understory for $\geq 50$ years. Previous studies have also shown little to no impact of invaders on soil C pools (Ehrenfeld, 2003; Hook et al., 2004). We also hypothesized that the lack of change in soil C pools would be a function of new grass-derived $C_4$-C replacing the net loss of forest $C_3$-C following invasion, as seen in other studies dealing with conversion of forests to pasture and cropland (Osher et al., 2003) and reforestation of abandoned cropland (Bashkin & Binkley, 1998; Binkley & Resh, 1999). Such replacement was evident in our study for the litter layer and fine root C pools, where net increases in $C_4$-C compensated for net losses of $C_3$-C (Fig. 7). However, these two components represent a small fraction of the total soil C pool. In contrast, for the very large mineral soil fine fraction pool, we found essentially no $C_4$-C (Fig. 7), suggesting that either belowground inputs of $C_4$-C are too small relative to the total mineral soil C pool or that $C_4$-C inputs are primarily to rapidly cycling pools that preclude stabilization in soil organic matter.

Differences between our results and prior studies (Bashkin & Binkley, 1998; Binkley & Resh, 1999; Osher et al., 2003) are likely due to differences in soils and the scope of land cover change encompassed in each study. Prior studies were conducted on deep ash soils with high clay content, while soils in this study contained little clay and $>2 \times$ the amount of organic C. In addition, this study examined the impact of grass invasion into intact forests, while prior studies dealt with more pronounced changes in land cover (e.g. forest to cropland).

**Soil C fluxes and $C_4$ contribution**

In agreement with the second hypothesis (H2), $R_{soil}$ was higher in grass-invaded plots (Fig. 1, Table 3). Increased $R_{soil}$ in grass plots was related to changes in total stand LAI (tree + understory) following invasion (Fig. 4). This result corroborates the increasingly common finding that $R_{soil}$ is tightly linked to canopy photosynthetic activity (Campbell et al., 2004; Irvine et al., 2005). We attribute higher $R_{soil}$ in grass-invaded plots to both greater LAI (Table 1) and enhanced litter decomposition (Fig. 6). Increased LAI would enhance the photosynthetic capacity of the system, while faster decomposition would shorten mean turnover time of soil C.

Our measurements of C fluxes into and out of soils do not take into account C that is allocated belowground by plants (total belowground C flux, or TBCF; Litton et al., 2007), but because $R_{soil}$ is the dominant component of the mass balance equation for estimating TBCF (Giardina & Ryan, 2002; Litton et al., 2004), increased $R_{soil}$ indicates that more C is allocated belowground in grass plots than in removal plots. However, our data also indicate that the potential increase of TBCF with invasion has not led to increased soil C storage. Instead, it appears that the majority of C allocated belowground in grass-invaded plots is rapidly returned to the atmosphere as $R_{soil}$ because: (i) grass-derived litter decomposes very rapidly (Fig. 6); (ii) grass-derived litter and mineral soil C are not accumulating (Table 2; Fig. 7); and (iii) $R_{soil}$ is composed largely of $C_4$-C where grasses occur ($>75$%; Fig. 7). Grass litter simply appears to decompose too rapidly and completely to be stabilized in mineral soil. In addition, we found no effect of grass invasion on the size of any soil C pool despite higher litterfall inputs, litter decomposition, and, presumably, belowground C flux in grass plots. However, it would likely take a very large change in C inputs to elicit a short-term response in pools (Smith, 2004).

As in previous studies (Yuste et al., 2003; Vasconcelos et al., 2004; Irvine et al., 2005), discrete precipitation pulses greatly increased $R_{soil}$ within 24 h (Fig. 3). Because efflux continued to increase for $>72 h$ after the pulse event, we attribute the high efflux rates following precipitation to enhanced microbial and root activity rather than flushing of CO$_2$ from soil air pores. Prior studies have shown strong and sustained increases in soil microbial activity and soil CO$_2$ release following pulse precipitation events (e.g. Miller et al., 2005).

Ignoring the effects of pulse events on cumulative flux calculated from linear interpolation between monthly measurements in this study resulted in large underestimates of efflux over 30–60 days. In contrast, ignoring discrete pulses had a much smaller impact on annual $R_{soil}$ estimates (Table 3). Borken et al. (2002) also found that $R_{soil}$ responded rapidly to soil rewetting in forest ecosystems, and that the transient increase in efflux was a small portion of annual $R_{soil}$. In contrast, other studies have shown that increased flux of $R_{soil}$ following pulse events can account for a significant portion of annual soil CO$_2$ efflux (Lee et al., 2004; Misson et al., 2006).

Contrary to the second part of H2, that the grass contribution to soil C fluxes would be proportional to its contribution to ecosystem C pools, we found that $R_{soil}$ was primarily of $C_4$ origin in grass plots (77%) despite grasses accounting for a small percentage of ecosystem C ($\sim 5$%; Litton et al., 2006). Interestingly, our 77% estimate of $C_4$ contribution to $R_{soil}$ closely matches the fraction of fine roots attributed to grasses (72%) and the $C_4$ contribution to aboveground litterfall in grass plots averaged over both years (75%). We interpret this as further evidence that grass-derived soil C inputs are
rapidly cycled and returned to the atmosphere. We also found that there was a small C_4 contribution to R_{soil} in removal plots (~10%; Fig. 7). The C_4 isotopic signature of R_{soil} in removal plots may be related to residual root C_4-C (Fig. 7). However, 10% is close to the overall resolution of the method, and designation of end member values and estimation errors associated with the Keeling plot method could account for most, if not all, of this value in removal plots.

Our estimate of the C_4 contribution to R_{soil} was also based on only one measurement period when \Psi_{soil} was \(-1.25\) MPa. This may not coincide with the C_4 contribution to cumulative annual R_{soil} as the \(\delta^{13}C\) signature of R_{soil} appears to be sensitive to soil moisture (Pregitzer et al., 2006) and \Psi_{soil} varied from \(-0.01\) to \(-7.11\) MPa in this study. Still, the grass contribution to R_{soil} was substantial despite the fact that grass is a very small fraction of ecosystem C.

Variability in precipitation

Considerable uncertainty resides in predictions of future tropical precipitation regimes, with models predicting both increased and decreased rainfall over the next 100 years for different parts of the equatorial Pacific (Meehl et al., 2005; Neelin et al., 2006). It is, thus, important to understand the impacts of changes in precipitation on belowground C dynamics. In line with our third hypothesis (H3), we found that increased precipitation enhanced the flux of C into and out of soils. However, we also hypothesized that higher precipitation would increase flux across all components and that the increase in flux would be relatively higher in grass plots. In contrast, we found that increased precipitation did not alter tree litterfall (Fig. 5), and that the relative increase in R_{soil} was the same for both treatments (~36%; Table 3). Grass litterfall, on the other hand, increased by 170% with higher precipitation (Fig. 5), increasing the C_4 contribution to total aboveground litterfall from 65% in Year 1 to 85% in Year 2 (Fig. 7).

Still, the litter layer C pool was smaller in Year 2, suggesting that decomposition in this system is primarily limited by moisture availability. Together these results indicate that R_{soil} will increase and continue to be dominated by C_4-C in grass-invaded forests if precipitation increases.

Generality of results

How might these results be influenced by the use of removal plots as a proxy of preinvasion conditions? Diaz et al. (2003) characterized the removal effect (i.e. the effect of removing an organism on a measured variable) as consisting of three facets: (i) how the system works when a particular organism is absent, (ii) response of remaining organisms, or colonizing organisms, to the removal, and (iii) the effect of the actual removal itself on physical, chemical and biotic components of the ecosystem (‘removal artifact’). The first two components of the removal effect were directly examined in this experiment – that is, how the suite of native species responded to and how ecosystem variables changed when an invasive grass was no longer present in the system. The third component of the removal effect was minimized in this study – the removal consisted of application of grass-specific herbicide, weed whacking, and manual extraction of aboveground grass biomass. Removal had no physical impact on the soil surface and, importantly, it occurred 3 years before the initiation of the experiment in a tropical system with year-round growing conditions.

What was the composition of the native understory before grass invasion, and what was its potential contribution to belowground C cycling? Very little is known about the native understory composition and structure in this system. Some insight, however, can be gained from an adjacent forest where P. setaceum has been removed for >10 years (Cabin et al., 2000). In this site, native shrubs, vines and herbaceous species have recolonized the understory. These are the same species that have colonized the removal plots. Moreover, the understory at a nearby forest fragment that has never been heavily invaded by P. setaceum is very similar in composition and structure to that in our removal plots (Litton et al., 2006).

Management and conservation implications

Future changes in global land cover and land use are uncertain, but are nonetheless predicted to have a large impact on the terrestrial C balance (Levy et al., 2004). In Hawaii, for example, most tropical dry forest has already been converted to non-native grassland (Bruegmann, 1996). In addition, once forests are invaded by P. setaceum, natural regeneration of native species all but ceases, indicating that even remaining forest fragments will eventually be converted (Litton et al., 2006). What happens to belowground C cycling following conversion of forests to grasslands? Given the large pools of soil C in this system, small changes in storage following conversion could have large implications for the regional flux of CO_2 to the atmosphere. Prior studies in this system have shown that elimination of the forest canopy and conversion to non-native grasslands in the presence of grazing reduces soil C pools (Elmore & Asner, 2006). Several lines of evidence support a strong role of grass invasion in this reduction of soil C. First, we found virtually no stabilization of grass C in soil.
organic matter in invaded plots. Second, $R_{\text{soil}}$ in converted grasslands dominated by \textit{P. setaceum} is up to 25% higher than in uninvaded forests (K. Zimmerman & C. M. Litton, unpublished data). Third, removal of the forest overstory will cease $C_3$ inputs of C into soils. Thus, high C flux rates out of soils in the absence of inputs into long-lived soil organic matter pools (e.g. wood) could explain the loss of soil C following conversion to non-native grasslands documented by Elmore & Asner (2006).

Predicting the impacts of invasive species on belowground C cycling is important for management and conservation globally, as soils are the largest sink and the second largest source of C in terrestrial ecosystems. Invasive species are already widespread, recent invasions will likely increase in coverage, and new invasions will undoubtedly occur. While this is certainly a challenging prospect, $R_{\text{soil}}$ is a good overall indicator of soil C dynamics. It is the dominant component of ecosystem respiration (Bolstad et al., 2004; Curtis et al., 2005; Yuste et al., 2005), and can be the ultimate determinant of the overall C balance in terrestrial ecosystems (Valentini et al., 2000). In this study, there was a strong positive correlation between annual $R_{\text{soil}}$ and total LAI, supporting a growing body of studies highlighting the importance of canopy processes in driving $R_{\text{soil}}$ (Ekblad & Högberg, 2001; Högberg et al., 2001; Edwards et al., 2004; Ekblad et al., 2005; Tang et al., 2005). This suggests that total stand LAI (overstory + understory), a commonly measured attribute of forest ecosystems, could be a useful indicator for assessing the impacts of invasive species on the most important flux of C from forests. Such an indicator would be particularly useful where estimates of changes in soil C pools are also available.

Conclusions

Understanding the impacts of invasive plant species on C cycling is crucial for predicting how invasions will impact the flux of C across the soil–plant–atmosphere interface and, thus, future atmospheric composition and climate. Earlier studies have primarily attributed changes in belowground C cycling following invasion to increased biomass (Ehrenfeld, 2003). Grass invasion into a forested ecosystem, then, would be expected to have negligible impacts on soil C pools and fluxes owing to its low biomass relative to total forest biomass. To the contrary, we found that grass invasion into a Hawaiian tropical dry forest substantially enhanced the flux of C into and out of soils. However, we observed negligible stabilization of grass-derived C in soil organic matter, and increased soil C fluxes did not change the size of any soil C pool. Greatly elevated rates of $R_{\text{soil}}$ with grass invasion are of particular concern given the importance of this flux in determining overall ecosystem C balance. If invasion ultimately leads to the conversion of forests to grasslands (Litton et al., 2006) and conversion and grazing lead to long-term decreases in soil C storage (Elmore & Asner, 2006), then non-native grass invasion into the understory of this forest could eventually release a large amount of sequestered soil C to the atmosphere. Regardless, it is clear that while grass invasion into forested areas in this system greatly increases soil C flux, as long as the forest canopy remains intact loss of soil C is unlikely.

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